IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING NATIONAL PHASE OF PCT APPLICATION UNDER 35 U.S.C. 371 AND 37 CFR 1.494 OR 1.495

To: Hon. Commissioner of Patents
Washington, D.C. 20231

00909

INITIAL LETTER TO THE UNITED STATES ATED/ELECTED OFFICE (DO/EO/US)

Atty Dkt: P 0282829 /SMC 60344/UST
M# /Client Ref.

Pillsbury Winthrop LLP, IP Group:

Date: August 30, 2001

This is a **REQUEST** for **FILING** a PCT/USA National Phase Application based on:

1. International Application	2. International Filing Date	3. Earliest Priority Date Claimed
PCT/GB00/00965	15 March 2000	19 March 1999
⇧ country code	Day MONTH Year	Day MONTH Year
		(use item 2 if no earlier priority)

4. Measured from the earliest priority date in item 3, this PCT/USA National Phase Application Request is being filed within:

(a) ☐ 20 months from above item 3 date (b) ☒ 30 months from above item 3 date,

(c) Therefore, the due date (unextendable) is September 19, 2001

5. Title of Invention 2'-SUBSTITUTED RNA PREPARATION

6. Inventor(s) REESE, Colin Bernard et al

Applicant herewith submits the following under 35 U.S.C. 371 to effect filing:

7. ☒ Please immediately start national examination procedures (35 U.S.C. 371 (f)).

8. ☒ **A copy of the International Application** as filed (35 U.S.C. 371(c)(2)) is transmitted herewith (file if in English but, if in foreign language, file only if not transmitted to PTO by the International Bureau) including:

a. ☒ Request;
b. ☒ Abstract;
c. 10 pgs. Spec. and Claims;
d. _____ sheet(s) Drawing which are ☐ informal ☐ formal of size ☐ A4 ☐ 11"

9. ☒ **A copy of the International Application has been transmitted by the International Bureau.**

10. **A translation of the International Application** into English (35 U.S.C. 371(c)(2))
a. ☐ is transmitted herewith including: (1) ☐ Request; (2) ☐ Abstract;
(3) _____ pgs. Spec. and Claims;
(4) _____ sheet(s) Drawing which are:
☐ informal ☐ formal of size ☐ A4 ☐ 11"
b. ☐ is not required, as the application was filed in English.
c. ☐ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.
d. ☐ Translation verification attached (not required now).

11. ☒ Please see the attached Preliminary Amendment

12. ☐ Amendments to the claims of the International Application **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., before 18th month from first priority date above in item 3, are transmitted herewith (file only if in English) including:**

13. ☒ PCT Article 19 claim amendments (if any) have been transmitted by the International Bureau

14. ☐ Translation of the amendments to the claims **under PCT Article 19 (35 U.S.C. 371(c)(3)), i.e., of claim amendments** made before 18th month, **is attached (required by 20th month from the date in item 3 if box 4(a) above is X'd, or 30th month if box 4(b) is X'd, or else amendments will be considered canceled).**

15. **A declaration of the inventor** (35 U.S.C. 371(c)(4))
a. ☐ is submitted herewith ☐ Original ☐ Facsimile/Copy
b. ☒ is not herewith, but will be filed when required by the forthcoming PTO Missing Requirements Notice per Rule 494(c) if box 4(a) is X'd or Rule 495(c) if box 4(b) is X'd.

16. **An International Search Report (ISR):**
a. Was prepared by ☒ European Patent Office ☐ Japanese Patent Office ☐ Other
b. ☒ has been transmitted by the international Bureau to PTO.
c. ☒ copy herewith (2 pg(s).) ☒ plus Annex of family members (1 pg(s).).

17. **International Preliminary Examination Report** (IPER):
a. ☒ has been transmitted (if this letter is filed after 28 months from date in item 3) in English by the International Bureau with Annexes (if any) in original language.
b. ☒ copy herewith in English.
c.1 ☐ IPER Annex(es) in original language ("Annexes" are amendments made to claims/spec/drawings during Examination) including attached amended:
c.2 ☐ Specification/claim pages #_____ claims #_____
Dwg Sheets #_____
d. ☐ Translation of Annex(es) to IPER, **(required by 30th month due date, or else annexed amendments will be considered canceled).**

18. **Information Disclosure Statement** including:
a. ☒ Attached Form PTO-1449 listing documents
b. ☒ Attached copies of documents listed on Form PTO-1449
c. ☒ A concise explanation of relevance of ISR references is given in the ISR.

19. ☐ **Assignment** document and Cover Sheet for recording are attached. Please mail the recorded assignment document back to the person whose signature, name and address appear at the end of this letter.

20. ☐ Copy of Power to IA agent.

21. ☐ **Drawings** (complete only if 8d or 10a(4) not completed): ___ sheet(s) per set: ☐ 1 set informal; ☐ Formal of size ☐ A4 ☐ 11"

22. Small Entity Status ☒ is **Not** claimed ☐ is claimed (**pre**-filing confirmation required)
22(a) _____ (No.) Small Entity Statement(s) enclosed (since 9/8/00 Small Entity Statements(s) not essential to make claim)

23. **Priority** is hereby claimed under 35 U.S.C. 119/365 based on the priority claim and the certified copy, both filed in the International Application during the international stage based on the filing in (country) GREAT BRITAIN of:

	Application No.	Filing Date		Application No.	Filing Date
(1)	9906328.1	March 19, 1999	(2)		
(3)			(4)		
(5)			(6)		

a. ☒ See Form PCT/IB/304 sent to US/DO with copy of priority documents. If copy has not been received, please proceed promptly to obtain same from the IB.
b. ☐ Copy of Form PCT/IB/304 attached.

RE: USA National Phase Filing of PCT/GB00/00965

24. Attached:

25 Per Item 17.c2, **cancel original** pages #_____, claims #_____, Drawing Sheets #_____

26. **Calculation of the U.S. National Fee (35 U.S.C. 371 (c)(1)) and other fees is as follows:**
Based on amended claim(s) per above item(s) ☐ 12, ☐ 14, ☐ 17, ☐ 25 (hilite)

Total Effective Claims	minus 20 =	x $18/$9 =	$0	966/967
Independent Claims	minus 3 =	x $80/$40 =	$0	964/965
If any proper (ignore improper) Multiple Dependent claim is present,		add$270/$135	+0	968/969

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(4)): →→ **BASIC FEE REQUIRED, NOW** →→→→

A. If country code letters in item 1 are **not** "US","BR","BB","TT","MX","IL" "NZ", "IN" or "ZA"

See item 16 re:

1.	Search Report was not prepared by EPO or JPO	add$1000/$500		960/961
2.	Search Report was prepared by EPO or JPO	add$860/$430	+860	970/971

SKIP B, C, D AND E UNLESS country code letters in item 1 are "US","BR","BB","TT","MX","IL", "NZ", "IN" or "ZA"

→ ☐ B.	If USPTO did not issue both International Search Report (ISR) and (if box 4(b) above is X'd) the International Examination Report (IPER),	add$1000/$500	+0	960/961
(X) (only) (one) → ☐ C.	If USPTO issued ISR but not IPER (or box 4(a) above is X'd),	add$710/$355	+0	958/959
(of these) (4) → ☐ D.	If USPTO issued IPER but IPER Sec. V boxes not all 3 YES, (boxes)	add$690/$345	+0	956/957
→ ☐ E	If international preliminary examination fee was paid to USPTO and Rules 492(a)(4) and 496(b) satisfied (IPER Sec. V all 3 boxes YES for all claims),	add $100/$50	+0	962/963
27		**SUBTOTAL =**	$860	
28.	If Assignment box 19 above is X'd, add Assignment Recording fee of	----$40	+0	(581)
29.	If box 15a is x'd, determine whether inventorship on Declaration is different than in international stage. If yes, add (per Rule 497(d)	----$130	+0	(098)
30.	Attached is a check to cover the	**TOTAL FEES**	$860	

Our Deposit Account No. 03-3975
Our Order No. 070662 (C#) | 0282829 (M#)

00909

CHARGE STATEMENT: The Commissioner is hereby authorized to charge any fee specifically authorized hereafter, or any missing or insufficient fee(s) filed, or asserted to be filed, or which should have been filed herewith or concerning any paper filed hereafter, and which may be required under Rules 16-18 and 492 (missing or insufficient fee only) now or hereafter relative to this application and the resulting Official document under Rule 20, or credit any overpayment, to our Account/Order Nos. shown above for which purpose a duplicate copy of this sheet is attached.
This CHARGE STATEMENT does not authorize charge of the issue fee until/unless an issue fee transmittal form is filed

Pillsbury Winthrop LLP
Intellectual Property Group

By Atty: Paul N. Kokulis **Reg. No. 16773**

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NOTE: File in duplicate with 2 postcard receipts (PAT-103) & attachments.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF

Inventor(s): REESE, Colin Bernard et al

Filed: Herewith

Title: 2'-SUBSTITUTED RNA PREPARATION

August 30, 2001

PRELIMINARY AMENDMENT

Hon. Commissioner of Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

IN THE SPECIFICATION:

At the top of the first page, just under the title, insert

☒ --This application is the National Phase of International Application PCT/GB00/00965 filed March 15, 2000 which designated the U.S. and that International Application

☒ was ☐ was not published under PCT Article 21(2) in English.--

Respectfully submitted,

PILLSBURY WINTHROP LLP
Intellectual Property Group

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09/914596
JC20 Rec'd PCT/PTO 3 0 AUG 2001

2'-SUBSTITUTED RNA PREPARATION

The present invention relates to a process for preparing 2'-O-substituted nucleosides, and more particularly to a process for the preparation of 2'-O-substituted uridine and cytidine.

The possibility that synthetic oligonucleotides might be effective inhibitors of gene expression and be used as chemotherapeutic agents has stimulated much research work in recent years. In order to avoid their degradation by cellular nucleases, it is essential that such oligonucleotides should be modified. Modifications can be made to the internucleotide linkages, the base residues and the sugar residues. A large number of oligonucleotide analogues in which the internucleotide linkages have been modified, especially as phosphorothioates with non-bridging sulphur atoms, have been described. Several of these phosphorothioate analogues are promising drug candidates that are now undergoing clinical trials. However, phosphorothioates do have some disadvantages. Thus, they do not display optimal RNA-binding properties and they also have a tendency to bind to proteins in a non-specific manner. Possible base modifications are clearly limited as they must not lead to a significant decrease in hybridisation properties. Recently, considerable interest has been directed towards the modification of the sugar residues. One particular type of modification involves the introduction of 2'-α-alkoxy groups (as in 2'-*O*-alkyl-oligoribonucleotides). While, in general, small alkoxy groups (such as methoxy) promote better hybridisation properties with complementary ribonucleic acids (RNA), nuclease resistance tends to increase with an increase in the size of the alkoxy group. 2-Methoxyethoxy has emerged as an alkoxy group that confers both good hybridisation properties and high nuclease resistance. It therefore seems likely that 2'-*O*-(2-methoxyethyl)-ribonucleosides will be incorporated into a second generation of potential oligonucleotide chemotherapeutic agents. For this reason, the development of convenient methods for the preparation of 2'-*O*-(2-methoxyethyl)-ribonucleosides has become a matter of much importance.

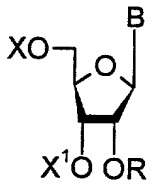
The preparation of 2'-*O*-(2-methoxyethyl)-ribonucleosides, starting from D-ribose, has previously been described. These preparations involved the use of protecting groups and required a relatively large number of steps. For example, 2'-*O*-(2-methoxyethyl)-5-methyluridine was prepared by Martin, P. Helv. Chim. Acta 1995, 78, 486-504 from D-ribose in 10 steps and in 33% overall yield. A later report by McGee and Zhai in Abstracts of American Chemical Society National Meeting, Division of Organic Chemistry, March 1996 paper 253 revealed a much more convenient procedure for the preparation of 2'-*O*-alkyl derivatives of the main pyrimidine ribonucleosides. Thus, when 5'-*O*-(4,4'-dimethoxytrityl)-2,2'-anhydro-1-β-D-arabinofuranosyluracil was heated with magnesium methoxide in N,N-dimethylformamide (DMF) at 100°C, 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-methyluridine was obtained in 94% yield. Somewhat lower yields of the corresponding 5'-

O-ethyl-, 5'-*O*-(n-propyl)- and 5'-*O*-allyl-uridine derivatives were obtained in the reactions between the same substrate and the appropriate magnesium alkoxides. It was also reported that magnesium alkoxides could be replaced by calcium alkoxides.

Ross et al reported in Nucleosides and Nucleotides, 1997, 16, 1641-3 that when unprotected 2,2'-anhydro-1-β-D-arabinofuranosyluracil was heated with a twofold excess of trimethyl borate and a stoichiometric quantity of trimethyl orthoformate in methanol at 150°C, under pressure, for 42 h, 2'-*O*-methyluridine was obtained in 86% isolated yield. 2'-*O*-Methyl-5-methyluridine was similarly prepared from 2,2'-anhydro-5-methyl-(1-β-D-arabinofuranosyluracil) by the borate ester procedure and, although no experimental details were provided, the preparation of 2'-*O*-methylcytidine was also reported. The yield of 2'-*O*-alkyl-uridine was stated to decrease with increasing alcohol size.

It remains desirable to identify additional or alternative routes for the preparation of 2'-O-substituted nucleosides.

According to the present invention, there is provided a process for the preparation of a compound of formula (1):



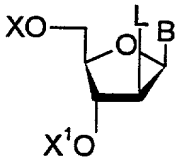
wherein:

X, and X' are each independently H or a protecting group;

B is a base; and

R is an alkyl, alkoxyalkyl, alkenyl, or alkynyl group, each of which may be optionally substituted;

which comprises reacting a compound of formula (2):



wherein

L is a leaving group; and

B, X and X' are as defined above

with a compound of formula $Al(OR)_3$ wherein R is as defined above, under substantially anhydrous conditions.

When R is alkenyl, the alkenyl group is often a C_{1-4} alkenyl group, especially an allyl or crotyl group. When R represents alkyl, the alkyl group is preferably a C_{1-4} alkyl, and most preferably a methyl or ethyl group. When R represents alkoxyalkyl, the alkoxyalkyl group is often a C_{1-4} alkyoxyC_{1-4} alkyl group, and preferably a methoxyethyl group. When R is alkynyl, the alkynyl group is often a C_{1-4} alkynyl group, especially a

propargyl group. The alkyl, alkenyl, alkynyl and alkoxyalkyl groups may themselves be substituted by one or more substituents, particularly halogen, especially F, Cl or Br, and amino substituents.

Examples of protecting groups which can be represented by X and X' include acid labile protecting groups, particularly trityl and substituted trityl groups such as dimethoxytrityl and 9-phenylxanthen-9-yl groups; acid-labile acetal protecting groups, particularly 1-(2-fluorophenyl)-4-methoxypiperidine-4-yl (Fpmp); and base labile-protecting groups such as acyl groups, commonly comprising up to 16 carbon atoms, such as ethanoyl groups or fatty alkanoyl groups, including particularly linear or branched C_{6-16} alkanoyl groups, such as lauroyl groups; benzoyl and substituted benzoyl groups, such as alkyl, commonly C_{1-4} alkyl-, and halo, commonly chloro or fluoro, substituted benzoyl groups.

Other suitable protecting groups include those derived from gamma keto acids, such as levulinoyl groups and substituted levulinoyl groups. Substituted levulinoyl groups include 5-halo-levulinoyl, such as 5,5,5-trifluorolevulinoyl and benzoylpropionyl groups; and silyl and siloxane ethers, such as alkyl, commonly C_{1-4} alkyl, and aryl, commonly phenyl, silyl ethers, particularly trialkylsilyl groups, often tri(C_{1-4}-alkyl)silyl groups, such as tertiary butyl dimethyl silyl and tertiary butyl diphenyl silyl groups.

Bases which can be represented by B include nucleobases, particularly purines, especially adenine (A) and guanine (G); and pyrimidines, especially thymine (T), cytosine (C), and uracil (U); and substituted derivatives thereof. Examples of substituents which may substitute the bases, in addition to protecting groups, include alkyl, especially C_{1-4}-alkyl, particularly methyl; halogen, particularly Cl or Br; amino; alkenyl, especially C_{1-4}-alkenyl and particularly allyl; alkoxyalkyl, especially C_{1-4}alkoxyC_{1-4}alkyl, particularly methoxyalkyl; and alkynyl, particularly propargyl, substitutents. The alkyl, alkenyl, alkynyl and alkoxyalkyl groups may themselves be substituted by one or more substituents, particularly halogen, especially F, Cl or Br, and amino substituents.

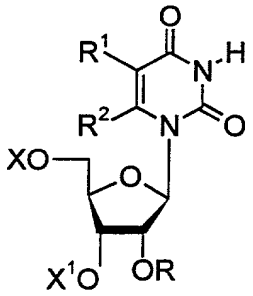
In addition to the presence of protecting groups X and X', bases employed in present invention may also be protected where necessary by suitable protecting groups. Protecting groups employed are those known in the art for protecting such bases. For example, A and/or C can be protected by benzoyl, including substituted benzoyl, for example alkyl- or alkoxy-, often C_{1-4} alkyl- or C_{1-4}alkoxy-, benzoyl; pivaloyl; and amidine, particularly dialkylaminomethylene, preferably di(C_{1-4}-alkyl) aminomethylene such as dimethyl or dibutyl aminomethylene. G may be protected by a phenyl group, including substituted phenyl, for example 2,5-dichlorophenyl and also by an isobutyryl group. T and U generally are not protected, but in certain embodiments they may advantageously be protected, for example at O4 by a phenyl group, including substituted phenyl, for example 2,4-dimethylphenyl or at N3 by a pivaloyloxymethyl, benzoyl, alkyl or alkoxy substituted benzoyl, such as C_{1-4} alkyl- or C_{1-4} alkoxybenzoyl.

In certain embodiments, X and X' comprise a single protecting group which protects both the 3' and 5' positions. Examples of such groups include disiloxanes, especially tetraalkyldisiloxanes, such as tetraisopropyldisiloxane.

Leaving groups which can be represented by L include those leaving groups which can be displaced by a nucleophile of formula RO^-. Examples of preferred leaving groups include groups of formula $-OSO_2CH_3$, $-OSO_2CF_3$, Cl, Br, I, O-Mesyl, O-Brosyl and O-Tosyl groups.

In certain preferred embodiments, the leaving group comprises the base, B, chemically bonded to the 2'-position, commonly via an oxygen or sulphur atom or a group of formula $-NR^x-$, wherein R^x is H or a C_{1-6} alkyl or aryl, such as a phenyl, group. Most preferably, the base is uracil bonded to the 2'-position via an oxygen atom.

Accordingly, a second aspect of the present invention provides a process for the preparation of a compound of formula (3):



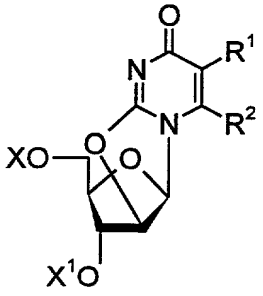
wherein:

X and X' are as defined above;

R^1 and R^2 are each independently H, alkyl, alkenyl, alkynyl, or halogen; and

R is an alkyl, alkoxyalkyl, alkenyl, or alkynyl group, each of which may be optionally substituted;

which comprises the reaction of a compound of formula (4)



wherein

X, X', R^1 and R^2 are as defined above;

with a compound of formula $Al(OR)_3$ wherein R is as defined above, under substantially anhydrous conditions.

When either of R^1 and R^2 is alkenyl, the alkenyl group is often a C_{1-4} alkenyl group, especially an allyl or crotyl group. When either of R^1 and R^2 represents alkyl, the alkyl group is preferably a C_{1-4} alkyl, and most preferably a methyl or ethyl group. When either

of R^1 and R^2 represents alkoxyalkyl, the alkoxyalkyl group is often a C_{1-4} alkyoxyC_{1-4} alkyl group, and preferably a methoxyethyl group. When either of R^1 and R^2 is alkynyl, the alkynyl group is often a C_{1-4} alkynyl group, especially a propargyl group. The alkyl, alkenyl and alkynyl groups represented by R^1 or R^2 may be substituted by one or more substituents, particularly halogen, especially F, Cl or Br, and amino substituents. When either of R^1 and R^2 is halogen the halogen is preferably Cl, Br or I. Most preferably, both of R^1 and R^2 represent H, or R^1 represents C_{1-4} alkyl and R^2 represents H.

The process according to the present invention takes place in the presence of a suitable substantially anhydrous solvent. Examples of suitable solvents include halocarbons such as chloroform, 1,2-dichloroethane and chlorobenzene; esters, particularly alkyl esters such as ethyl acetate, and methyl or ethyl propionate; amides such as N-methylpyrrolidinone, dimethylformamide and particularly dimethylacetamide; lower alkyl, for example C_{2-4} nitriles such as acetonitrile; ethers such as glyme and diglyme and cyclic ethers such as tetrahydrofuran and dioxane; tertiary amines, such as N-methylpyrrolidine and heterocyclic aromatic amines such as pyridine. and alcohols, most commonly the alcohol corresponding to the group R, for example methanol, ethanol, methoxyethanol, allyl alcohol or propargyl alcohol.

The process of the present invention is often carried out at a temperature of from room temperature, such as about 25°C, up to the reflux temperature of the solvent employed. Temperatures above the normal boiling point of the solvent employed can be employed if desired by carrying out the process under super-atmospheric pressure conditions, for example in a sealed reaction vessel. Commonly, the temperature is in the range of from 50 to 150°C.

The process commonly takes place over a period ranging from several hours, for example from 4 to 12 hours, to several days, for example from 1 to 2 days, depending on the reagents and reaction conditions employed.

When the compound of formula (1) comprises the base uracil, the uracil moiety may be converted to a cytosine moiety. Similarly, the uracil moiety comprised in the compound of formula (3) may also be converted to a cytosine moiety. The skilled man will recognise that a number of different techniques can be employed. Examples of such techniques include:

a) the nitrophenyl route (see Miah et al, Nucleosides and Nucletides, 1997, 16, pp53-65) where for example the uracil containing compound is reacted with chlorotrimethylsilane in acetonitrile/1-methylpyrrolidine, then with trifluoroacetic anhydride, followed by 4-nitrophenol. The 4-nitrophenol moiety is then displaced with ammonia in aqueous dioxane to yield the cytosine-containing compound; and

b) the triazolation procedure, (see Divakar et al, J. Chem. Soc. Perkin Trans. 1, 1982, 1171-6) where for example the uracil containing compound is reacted with acetic anhydride in pyridine, then, after work up, with phosphoryl chloride, 1,2,4-triazole and

triethylamine in acetonitrile to give the 4-triazolopyrimidine. The triazole moiety is then displaced with ammonia in aqueous dioxane, and acetyl groups removed to yield the cytosine-containing compound.

Protecting groups can be removed using methods known in the art for the particular protecting group and function. For example, acyl protecting groups, such as ethanoyl and benzoyl groups, can be removed by treatment with a solution of ammonia in an alcohol such as ethanol.

Benzoyl, pivaloyl and amidine groups can be removed by treatment with concentrated aqueous ammonia.

Trityl groups present can be removed by treatment with acid, for example a solution of dichloroacetic acid in dichloromethane. With regard to the overall unblocking strategy an important consideration is that the removal of trityl, often DMTr, protecting groups ('detritylation') should proceed without concomitant depurination when base B represents a purine, especially adenine. Such depurination can be suppressed by effecting 'detritylation' with a dilute solution of hydrogen chloride at low temperature, particularly *ca.* 0.45 M hydrogen chloride in dioxane - dichloromethane (1:8 v/v) solution at -50°C. Under these reaction conditions, 'detritylation' can be completed rapidly, and in certain cases after 5 minutes or less.

Silyl protecting groups may be removed by fluoride treatment, for example with a solution of a tetraalkyl ammonium fluoride salt such as tetrabutyl ammonium fluoride.

Fpmp protecting groups may be removed by acidic hydrolysis under mild conditions.

Compounds produced by the present invention may be incorporated in the assembly of a desired oligonucleotide by coupling with other nucleosides or oligonucleotides (which may themselves have been prepared using the present invention) and such a process forms a further aspect of the present invention. The coupling processes employed are those known in the art for the preparation of oligonucleotides.

The present invention is further illustrated, but not limited by, the following Examples.

General Experimental Details

Mps are uncorrected. ^{1}H and ^{13}C NMR spectra were measured at 360.1 and 90.6 MHz respectively, with a Bruker AM 360 spectrometer; tetramethylsilane was used as an internal standard. TLC was carried out with Merck silica gel 60 F_{254} pre-coated plates (Art 5715), which were developed in solvent system A [$CHCl_3$-MeOH (85:15 v/v)]. Short column chromotography was carried out on silica gel (Merck Art 7729). Acetonitrile and 1-methylpyrrolidine were dried by heating, under reflux, with calcium hydride and were then distilled. N,N-Dimethylacetamide (DMA) was dried by distillation over calcium hydride under reduced pressure. 2-Methoxyethanol was dried by heating with aluminium

foil (1g/250ml), under reflux, and was then distilled. Diethyl ether was dried over sodium wire.

Preparation of 2,2'-Anhydro-1-β-D-arabinofuranosyluracil

Uridine (12.21g, 50mmol), diphenyl carbonate (11.79g, 55mmol), sodium hydrogen carbonate (0.21g, 2.5mmol) and dry DMA (10ml) were heated together, with stirring, at 100°C. After 5h, the products were cooled to room temperature, and diethyl ether (100ml) was added with stirring. After 2 hours, the colourless precipitate (11.70g) was collected by filtration and was washed with ether (2 x 50ml). The sole nucleoside constituent of the precipitated material was identified as 2,2'-anhydro-1-β-D-arabinofuranosyluracil (calculated quantitative yield, 11.31g) by comparison with authentic material.

Preparation of 2'-*O*-(2-Methoxyethyl)uridine

Aluminium foil (3.64g, 0.135 mol) and dry 2-methoxyethanol (135ml) were heated, under reflux, for ca. 1hr until all of the aluminium had been consumed. Crude (see above) 2,2'-anhydro-1-β-D-arabinofuranosyluracil (10.18g, ca. 43.5 mmol) was added and the reactants were heated, under reflux, for 48 hours. Absolute ethanol (200ml), followed by water (7.3ml, 0.405mol) and Celite were added to the cooled products. The resulting mixture was heated, under reflux, for 10 minutes and was then filtered. The residue was washed with ethanol (3 x 100ml). The combined filtrate and washings were evaporated under reduced pressure to give a pale yellow solid. The material was purified by short column choromatography on silica gel (70g): the appropriate fractions, which were eluted with dichloromethane-methanol (90:10 v/v), were evaporated under reduced pressure to give the title compound as a colourless solid (12.05g, ca. 91%).

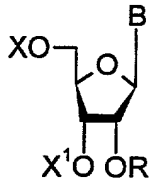
Preparation of 2'-*O*-(2-Methoxyethyl)cytidine

2'-*O*-(2-Methoxyethyl)uridine (6.05g, 20.0mmol), 1-methylpyrrolidine (20ml, 0.192mol), chlorotrimethylsilane (7.6ml, 59.9mmol) and dry acetonitrile (100ml) were stirred together at room temperature. After 1 hour, the reactants were cooled to 0°C (ice-water bath) and trifluoroacetic anhydride (7.1ml, 50.3 mmol) was added dropwise over 5 minutes. After a further period of 30 minutes at 0°C, 4-nitrophenol (8.35g, 60mmol) was added to the stirred reactants which were maintained at 0°C. After 3 hours, the products were poured into saturated aqueous sodium hydrogencarbonate (200ml), and the resulting mixture was extracted with dichloromethane (3 x 100ml). The combined organic layers were dried ($MgSO_4$), and evaporated under reduced pressure. Concentrated aqueous ammonia (d 0.88, 20ml) was added to a stirred solution of the residue in dioxane (100ml), contained in a sealed flask that was then heated at 55°C for 24 hours. The resulting yellow solution was concentrated under reduced pressure, and the residue was

evaporated with absolute ethanol (3 x 50ml). The products were fractionated by short column chromatography on silica gel: the appropriate fractions, which were eluted with dichloromethane-methanol-triethylamine (93:7:0.5 to 90:10:0.5 v/v) were evaporated under the reduced pressure to give the title compound as an off-white solid (5.07g 84%).

CLAIMS

1. A process for the preparation of a compound of formula (1):



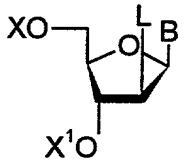
wherein:

X, and X' are each independently H or a protecting group;

B is a base; and

R is an alkyl, alkoxyalkyl, alkenyl, or alkynyl group, each of which may be optionally substituted;

which comprises reacting a compound of formula (2):



wherein

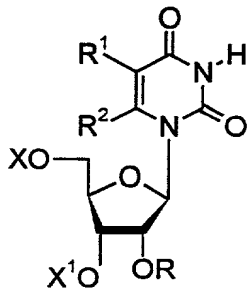
L is a leaving group; and

B, X and X' are as defined above

with a compound of formula $Al(OR)_3$ wherein R is as defined above, under substantially anhydrous conditions.

2. A process according to claim 1, wherein the leaving group is selected from the group consisting of $-OSO_2CH_3$, $-OSO_2CF_3$, Cl, Br, I, O-Mesyl, O-Brosyl, O-Tosyl and the base, B, chemically bonded to the 2'-position, via an oxygen or sulphur atom or a moiety of formula $-NR^x-$, wherein R^x is H or a C_{1-6} alkyl or an aryl group.

3. A process for the preparation of a compound of formula (3):



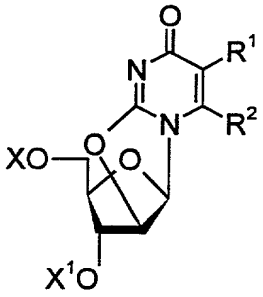
wherein:

X, and X' are each independently H or a protecting group;;

R^1 and R^2 are each independently H, alkyl, alkenyl, alkynyl, or halogen; and

R is an alkyl, alkoxyalkyl, alkenyl, or alkynyl group, each of which may be optionally substituted;

which comprises the reaction of a compound of formula (4)



wherein

X, X', R^1 and R^2 are as defined above;

with a compound of formula $Al(OR)_3$ wherein R is as defined above, under substantially anhydrous conditions.

4. A process according to claim 3, wherein R^1 and R^2 are both H, or R^1 is C_{1-4} alkyl, and R^2 is H.

5. A process according to any preceding claim, wherein R is a C_{1-4} alkenyl group, a C_{1-4} alkyl group, a C_{1-4} alkyoxyC_{1-4} alkyl group or a C_{1-4} alkynyl group.

6. A process according to claim 5, wherein R is a methoxyethyl group.

7. A process for the preparation of a compound of Formula (1) wherein B represents cytosine, or a substituted derivative thereof, which comprises:

a) preparing a compound of Formula (1) wherein B represents uracil, or a substituted derivative thereof, by a process according to claim 1; and

b) converting the uracil moiety to the equivalent cytosine moiety; or

c) preparing a compound of Formula (3) by a process according to claim 2; and

d) converting the uracil moiety therein to a cytosine moiety.

8. A process for the preparation of a product oligonucleotide which comprises the coupling to a nucleoside or an oligonucleotide of a compound prepared by a process according to any one preceding claim.

RULE 63 (37 C.F.R. 1.63)
DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the INVENTION ENTITLED:

2' substituted RNA preparation

the specification of which

is attached hereto
was filed on as U.S. application serial No.
X was filed as PCT international application No. PCT/GB00/00965 on 15/03/2000
and (if applicable) was amended on

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information which is known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate filed by me or my assignee disclosing the subject matter claimed in this application and having a filing date (1) before that of the application on which priority is claimed, or (2) if no priority is claimed, before the filing date of this application:

PRIOR FOREIGN APPLICATION(S)

Number	Country	Day/MONTH/Year Filed	Date First Laid Open or published	Date Patented or Granted	Priority claimed Yes	No
9906328.1	United Kingdom	19/03/1999			X	

I hereby claim the benefit under 35 U.S.C 120/365 of all United States applications listed below and PCT international applications listed above or below and, if this is a continuation-

in-part (CIP) application insofar as the subject matter disclosed and claimed in this application is in addition to that disclosed such in the prior applications. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in 37 C.F.R. 1.56 which became available between the filing date of each such prior application and the national or PCT international filing date of this application:

PRIOR U.S. OR PCT APPLICATION(S) Application No. (Serial Code/Serial No.) Day/MONTH/Year Filed	Status (patented, pending abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

And I hereby appoint Pillsbury Winthrop LLP, 1600 Tysons Boulevard, McLean, Virginia 22102 USA, telephone number 861-3000 (to whom all communications should be directed), and the below named persons (of the same address) individually and collectively my attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent and I hereby authorise them to act and reply on instructions from and communicate directly with the person/assignee/attorney/firm/ organisation who/which first sends/sent this case to them and by who/which I hereby declare that I have consented after full disclosure to be represented unless/until I instruct Pillsbury Winthrop in writing to the contrary.

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